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Comparative analysis of non-random DNA repair following *Ac* transposon excision in maize and *Arabidopsis*

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Summary

***Ac/Ds* transposable elements often leave short DNA rearrangements, or 'footprints,' at the sites where they excise. Previous studies at the maize *waxy* (*wx*) gene suggest that the DNA repair that forms transposon footprints is not random. Each excision site consistently displays a different, predominant repair product suggesting flanking DNA may influence footprint formation. We have expanded these studies to show that predominant end-joining products also form in association with *Ac/Ds* excision in *Arabidopsis* and that chromosomal location of the *Ac*-containing construct does not appear to influence this repair. The predominant repair product is identical in both maize and *Arabidopsis* for *Ac* elements with the same adjacent DNA sequences. However, a broader range of minor footprint types is observed in *Arabidopsis*, including footprints that are rare in maize, suggesting potential differences in the host proteins involved in either transposition, repair or both. The data also suggest that the sequences influencing footprint formation are within 39 bp 5′ and 18 bp 3′ of the transposon. These studies demonstrate that transgenic *Ac/Ds*-containing plants will be useful tools in dissecting plant DNA repair processes.**

Introduction

The transposable elements *Activator (Ac)* and *Dissociation (Ds)* can influence gene expression as well as alter genome structure in a variety of plant hosts (for review see Kunze, 1996). Excision of *Ac/Ds* elements can cause chromosome breaks, inversions and translocations (English *et al.*, 1993; English *et al.*, 1995; Dooner *et al.*, 1991; Doring *et al.*, 1989; McClintock, 1951; McClintock, 1956; Weil and Wessler, 1993); however, the most frequent results of *Ac/Ds* excision are small sequence alterations, or 'transposon footprints' at the excision site. Whether gene function is restored depends on the number and sequence of bases added or removed (Wessler, 1988); the original target sequence is restored only rarely (Baran *et al.*, 1992; Scott *et al.*, 1996).

Ac/Ds elements transpose via double-stranded DNA intermediates and footprints are the product of transposase-mediated cutting, followed by DNA end-joining repair either by transposase, by host repair functions or some combination of both. Little is known about the mechanisms involved or the transposon DNA topology during transposition, both of which are likely to affect the way in which the DNA is repaired. In addition, host plant genes are likely to be involved in transposition, an idea supported by reports of tobacco and maize proteins that bind subterminal regions of *Ac* (Becker and Kunze, 1996; Levy *et al.*, 1996) and recessive *Arabidopsis* mutations that increase *Ac* transposition (Jarvis *et al.*, 1997).

Interactions between *Ac/Ds* and its host probably vary among species (Altmann *et al.*, 1995; Bancroft and Dean, 1993; Carroll *et al.*, 1995; Dean *et al.*, 1992; Keller *et al.*, 1993; Lawson *et al.*, 1994; Robbins *et al.*, 1994; Schmidt and Willmitzer, 1989; Scofield *et al.*, 1992; Swinburne *et al.*, 1992; Van Sluys *et al.*, 1987; Yoder, 1990). However, one common feature is that *Ac/Ds* excision leaves transposon footprints in all species examined to date, suggesting at least some similarity in the host repair of *Ac/Ds*-induced damage (van der Biezen *et al.*, 1996; Pohlman *et al.*, 1984; Schmidt-Rogge *et al.*, 1994; Yang *et al.*, 1993). The majority of footprints studied have come from maize, where the eight base pair target site duplications flanking the transposon are often left intact except for one or two base pairs closest to the element; these bases are usually deleted, inverted or replaced by their complement (see Scott *et al.*, 1996). More extensive deletions also occur, and may even extend beyond the eight bp duplication, but these are rare in maize. Interestingly, extensive deletions into host sequences are more commonplace following excision of the *Ac*-related transposon *Tam3* in snapdragon (reviewed by Coen *et al.*, 1989), as well as the unrelated *Mutator* element in maize (reviewed by Chandler and Hardeman, 1992).

DNA end-joining repair accompanying *Ds* excision is non-random and flanking sequences appear to influence the repair product formed (Scott *et al.*, 1996). Excisions of one *Ds* from six different sites in the maize *waxy (wx)* gene were analysed using a PCR-based assay (Baran *et al.*, 1992; Britt and Walbot, 1991; Doseff *et al.*, 1991; Scott *et al.*, 1996), and repair products for each allele amplified, cloned and sequenced (Scott *et al.*, 1996). Surprisingly, for any given allele the same footprint was recovered more often than any other in each plant carrying that allele; it is

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interesting to note that the *Ac*-related *hobo* element in *Drosophila* may also leave one excision product more often than any other (Atkinson *et al.*, 1993; Handler *et al.*, 1995). The predominant *Ds* footprint at each of the six *wx* sites differed in the number of additional bases and sequence alterations found. A low level of excision products that restored the wildtype sequence (+0 excisions) were also recovered for each allele.

We examined whether *Ac/Ds* elements show the same, non-random footprint formation in *Arabidopsis,* a more tractable transgenic system for dissecting footprint formation. We also examined whether the predominant footprint was the same as in maize for a given flanking sequence, and whether position within the *Arabidopsis* genome affected the DNA repair following *Ac/Ds* excision. Here, we describe somatic footprints recovered from four *Arabidopsis* lines carrying single copies of a modified *Ac* element, each at a different chromosomal location (Bhatt *et al.*, 1996; Lawson *et al.*, 1994). The sequences immediately adjacent to each *Ac* element in these constructs are identical to those at the maize *wx-m7* allele (Muller-Neumann *et al.*, 1984), allowing us to compare directly products of excision from the same flanking DNA in both *Arabidopsis* and maize.

Results and discussion

Footprint formation for maize wx-m7

We examined somatic footprints left after *Ac* excision from the maize *wx-m7* allele (Muller-Neumann *et al.*, 1984) by PCR amplifying *wx-m7* genomic DNA using the '*131*' and '*Ex1*' primers (Figure 1), cloning and sequencing; this assay and its rationale have been described in detail previously (Scott *et al.*, 1996). Briefly, by cloning individual, amplified molecules and sequencing random clones we can estimate the types of footprints formed in a plant and the relative abundance of particular template molecules (representing particular footprints) in the pool of amplified products. We amplified, sequenced and compared footprints from at least three plants to reduce the chance that an *Ac* excision early in development could bias the data. In addition, PCR reactions on each template were performed in triplicate to minimize PCR founder effects or amplification anomalies.

A predominant footprint was recovered after *Ac* excision just as for the 2 kb *Ds6*-like element studied previously (Scott *et al.*, 1996). This footprint added eight extra base pairs to the wild type sequence ('+8'), and appears in >50% of the cloned footprints for each *wx-m7* plant tested (Figure 2). As in our *Ds* studies, the *Ac* footprints recovered from *wx-m7* are consistent with the idea that the base nearest the element at each end is deleted or replaced by its complement (Scott *et al.*, 1996). We recovered the same low level of clones with +0 excisions (1–2%) previously observed in our *Ds* studies.

Footprint formation in Arabidopsis

The two *Arabidopsis* lines, A2 and A3, each have a T-DNA insertion carrying an *Ac*Δ*Nae*I element in the 5′ UTL of a streptomycin phosphotransferase (*SPT*) marker (Figure 1; Lawson *et al.*, 1994). *Ac*Δ*Nae*I is derived from the maize *wx-m7* allele and these constructs have 39 bp of *wx* DNA flanking the *Ac* at its 5′ end and 18 bp of *wx* DNA at its 3′ end. *Arabidopsis* lines B3 and B10 have T-DNAs with insertions at the same position in the *SPT* gene, however the *Ac*Δ*Nae*I and flanking maize DNA are in the opposite orientation compared with the A2 and A3 lines (Figure 1; Lawson *et al.*, 1994). All four T-DNAs map to different locations in the *Arabidopsis* genome (Bhatt *et al.*, 1996).

Excision of *Ac*Δ*Nae*I from our *Arabidopsis* lines yielded, as in maize, one footprint most often in each line (Figure 3). The A2 and A3 lines consistently produce a predominant +8 footprint which is identical in sequence to the predominant footprint produced by the maize *wx-m7* allele. These footprints were not contaminants from the previous maize studies because they were flanked by *SPT* sequences (data not shown). The predominant footprint is found in more than half of the clones from all but one of the plants for both of the A2 and A3 lines. We suspect the exception, plant A3B, yielded a different predominant footprint (Figure 3) because a developmentally early excision event biased the footprints collected from this plant (see Scott *et al.*, 1996).

As shown in Figure 3, plants from the B3 and B10 lines consistently produced the same predominant +8 footprint we observed in the A2 and A3 lines; again, this footprint occurred in >50% of the clones for any one plant. All four *Arabidopsis* lines and the maize *wx-m7* allele have only 39 bp 5′ and 18 bp 3′ of the *Ac* in common, yet all yield the same predominant footprint. We therefore conclude that these flanking sequences are sufficient to determine the predominant footprint.

Our observation that damage caused by *Ac* excision is managed in a similar, non-random manner in both maize and *Arabidopsis* suggests that flanking DNA might affect end-joining in other systems. In support of this idea, flanking sequences have been shown to influence end-joining double-strand break (DSB) repair of V(D)J coding joints in mammalian immunoglobulin genes (Boubnov *et al.*, 1993; Meier and Lewis, 1993; Nadel and Feeney, 1995). Interestingly, these coding joints resemble transposon footprints and occur by resolution of hairpin structures formed at site-specific DSBs (van Gent *et al.*, 1996; Roth *et al.*, 1992), a mechanism first proposed for plant transposon footprints (Coen *et al.*, 1986) (see below and Figure 4).

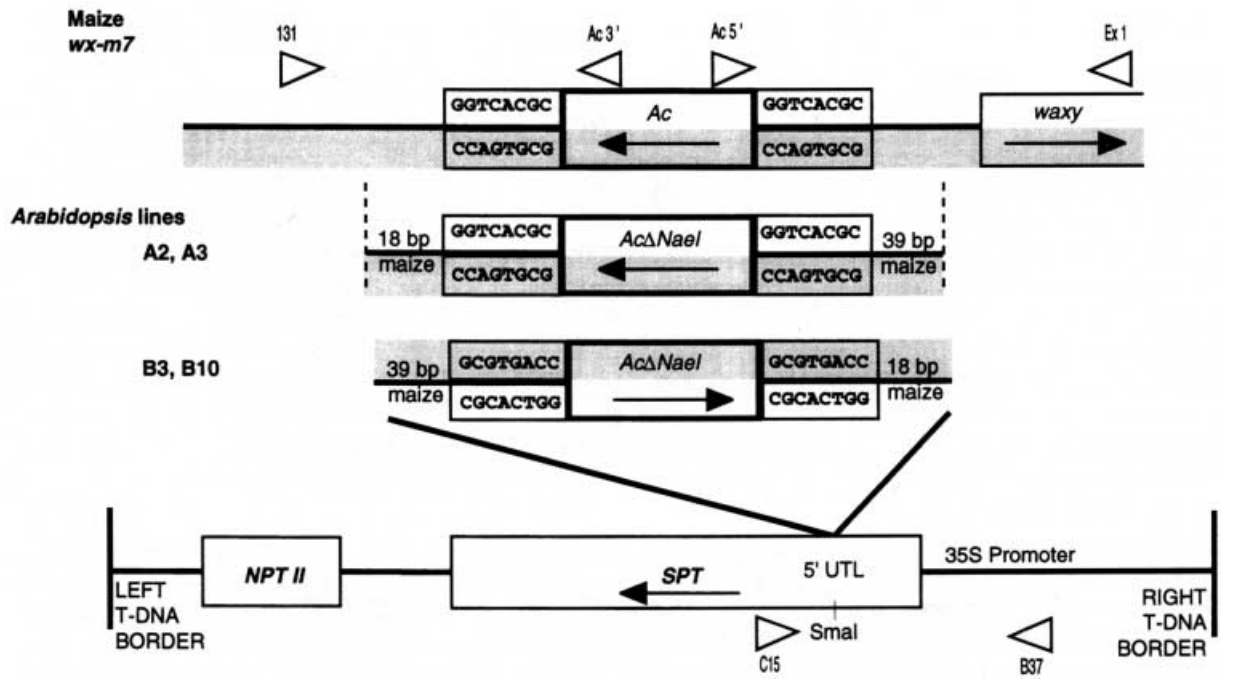


Figure 1. Maize *wx-m7* and *SPT::AcΔNaeI* constructs.
The direction of *wx* and *Ac* transcription are indicated by thin arrows. T-DNA constructs in the transgenic *Arabidopsis* A2 and A3 lines and B3 and B10 lines are diagrammed below. Genes are displayed as white boxes and orientation of transcription is indicated by arrows where relevant. *Ac* and adjacent maize sequences are inserted at the same site in the *SPT* 5′ UTL region for both constructs. PCR primers are indicated as open triangles. Primers *131* and *Ex1* were used to amplify repair products of *wx-m7* in maize. The *C15* and *B37* primers pictured below the T-DNA were used to amplify repair products following *AcΔNaeI* excision. *Ac3′* and *Ac5′* primers were used with appropriate primers in flankng DNA to verify the presence and orientation of *Ac* in each experiment.

wx-m7 (n=119)		Totals	Plants			
			A	C	D	
GGTCACGC	**GGTCACGC**					
GGTCACGg	cGTCACGC	**76**	**14**	**41**	**21**	**+8**
GGTCACG-	-GTCACGC	12	1	7	4	+6
GGTCACG-	ccGTCACGC	11	4	4	3	+8
GGTCACGg	ccGTCACGC	8		4	4	+9
GGTCACG-	--TCACGC	2	1	1		+5
GG------	ccGTCACGC	1	1			+3
GGTCACGgt	cgGTCACGC*	1		1		+10
GGTCACa	gGTCACGC*	1		1		+7
GGTCACGgcg	-GTCACGC	1	1			+9
GGTCACGgc	---CACGC	1	1			+6
GGTCACG-	--cCACGC	1	1			+5
GGTCACGg	----ACGC	1	1			+4
GGTCACGC		3	2		1	+0

Figure 2. *wx-m7* footprints from maize.
Repair products collected by PCR amplification and sequencing are organized into columns corresponding to individual plants examined. Complete target site duplications are in bold and repair products are listed below. Dashed lines indicate missing bases, base changes are shown in lowercase and bases that cannot be clearly assigned as changed are shown in lower case italics. Numbers correspond to the number of random clones sequenced which contain that particular sequence. *Footprint cannot be formed using the endonuclease model as proposed by Coen *et al.* (1986).

Influence of genomic location on footprint formation

The chromosomal location of *AcΔNaeI*-containing T-DNA constructs did not appear to influence whether or what type of predominant footprint was formed (Figure 3). In the A2 line, the *SPT::AcΔNaeI* construct maps to chromosome 4, in the A3 line to chromosome 5, in the B3 line to chromosome 2 and in the B10 line to a second location on chromosome 5 (Bhatt *et al.*, 1996). Furthermore, no apparent difference in the range of minor footprints formed can be attributed to genomic location in any of the four lines. This finding suggests that footprint formation from manipulated constructs transformed back into *Arabidopsis* would not be influenced significantly by T-DNA location.

Range of repair products formed

Ac excision from *wx-m7* yielded a total of 12 different footprints similar to those left by *Ac/Ds* at other sites in the *wx* gene (Scott *et al.*, 1996) and at a variety of *Ac/Ds*-induced alleles at other loci. The majority of these footprints were +6 or greater and no small footprints, e.g., +2, +1 or −1 were present. In addition, a low level of +0 events was observed and there were no deletions extending beyond the eight base pair target site duplications (Figure 2).

In striking contrast to footprints from *wx-m7* in maize, the *Arabidopsis* line A2 yielded 18 different minor footprints ranging in size from −12 to +9 while A3 yielded 17 minor footprints ranging from −10 to +9 (Figure 3). Among the products recovered from these lines were multiple clones with +2 and +1 footprints, which we have never recovered in our maize experiments; we also found a low but consist-

A2 (n=166)

		Totals	Plants			
			E	F	K	
GGTCACGC	**GGTCACGC**					
GGTCACGg	cGTCACGC	**106**	**30**	**39**	**37**	**+8**
GGTCACGcg	--------	15	15			+1
GGTCACG-	ccGTCACGC	8		7	1	+7
GGTCACGgcg	--------	7	7			+2
GGTCACG-	---CACGC	5			5	+5
GGTCA---	----ACGC	4			4	+1
GGTCACG-	cGTCACGC	4		4		+7
GGTCACGcg	---CACGC	2	2			+6
GGTCAtg-	gcGTCACGC*	2		1	1	+8
GGTCACGgc	--TCACGC	1	1			+7
GGTCAC--	-GTCACGC	1		1		+5
GGTCAC--	accGTCACGC	1		1		+8
GGTCACG-	---ctCGC*	1		1		+4
GGTCACG-	-----CGC	1	1			+2
GGTCACG-	>>(-11bp)	1			1	-12
GGTCACtg	---CACGC*	1			1	+5
GGTCtacgc	-------g#	1	1			+2
GGTCACGg	ccGTCACGC	1		1		+9
GGTCACGC		4		1	3	+0

A3 (n=204)

		Totals	Plants			
			A	B	E	F
GGTCACGC	**GGTCACGC**					
GGTCACGg	cGTCACGC	**125**	**52**		**41**	**32**	**+8**
GGTCACGg	---CACGC	27	2		2	23	+5
GGTCACGg	ccGTCACGC	25		25			+9
GGTCACGg	-------C	3			3		+1
GGTC----	--TCACGC	2		2			+2
GG------	-------C	2	2				-5
GGTCACG-	ccGTCACGC	2			1	1	+8
GGT-----	----ACGC	2	2				-1
GGTCACGgc	--TCACGC	2	1		1		+7
GGTCAC--	--TCACGC	1				1	+4
GGTCACG-	cGTCACGC	1			1		+7
GGTCACG-	-GTCACGC	1	1				+6
GG------	---CACGC	1				1	-1
GGTCACGgcgtcgc	-------g#	1	1				+7
GGTCACGgcgt	>>(-13bp)	1	1				-10
GGTCACGgc	----tCGC*	1				1	+5
(-5bp)<<	accGTCACGC	1				1	-3
GGTCACGC		6	1		5		+0

B3 (n=148)

		Totals	Plants			
			A	E	F	
GGTCACGC	**GGTCACGC**					
GGTCACGg	cGTCACGC	**100**	**29**	**31**	**40**	**+8**
GGT-----	----ACGC	10	8		2	-1
GGTCACG-	ccGTCACGC	8	5	1	2	+8
GGTCAC--	--TCACGC	7	3		4	+4
GGTCAC--	cGTCACGC	4	3		1	+6
GGTCACGg	---CACGC	4	1		3	+5
(-9bp)<<	-GTCACGC	3	2		1	-10
GGTCA---	--TCACGC	2	1	1		+3
GGTCACG-	------GC	2		2		+1
GGTCACGg	-----CGC	2	1		1	+3
GGTCACG-	-GTCACGC	1	1			+6
GGTCACG-	-----CGC	1			1	+2
GGTCACGg	----ACGC	1	1			+4
GGTCAC--	----ACGC	1		1		+2
GG------	---CACGC	1			1	-1
GGTCACGC		1			1	+0

B10 (n=135)

		Totals	Plants			
			B	G	I	
GGTCACGC	**GGTCACGC**					
GGTCACGg	cGTCACGC	**103**	**29**	**31**	**43**	**+8**
GGTCACG-	ccGTCACGC	6	5	1		+8
GGT-----	----ACGC	5		3	2	-1
GGTCACG-	--TCACGC	4	1	3		+5
t-------	--------	2			2	-7
GGTCAC--	ctgCACGC*	2			2	+6
GGTCACc-	---agCGC#	2	2			+4
GGTCACGc	---CACGC	1	1			+5
GGTCACGgc	>>(-21 bp)	1	1			-20
GGTCAC--	accGTCACGC	1			1	+7
GGTCACcg	---gtCGC*	1		1		+5
GGTCACGc	---gtCGC	1		1		+5
GGTCACcacgc	-------g#	1		1		+4
GGTCACGgcgtgacc	-GTCACGC	1			1	+14
GGTCACGC		4		2	2	+0

Figure 3. *AcΔNael* footprints from *Arabidopsis*.
Repair products collected and displayed as in Figure 2. For clarity, footprints recovered from the B3 and B10 lines are shown as their complements to permit direct comparison with those from the A2 and A3 lines. Deletions that extend beyond the target site duplications are indicated by >> or << and the total number of bases deleted from that side. *Footprint cannot be formed using the endonuclease model as proposed by Coen *et al.* (1986). #Footprint cannot be formed by either the endonuclease model or the exonuclease model proposed by Saedler and Nevers (1985) and modified by Scott *et al.* (1996).

ent level of deletion products (e.g., –1, –3, –4, etc.). Both the B3 and B10 lines also produced a wider range of footprints than maize, ranging from a highly unusual +14 sequence to a –20 deletion (Figure 3). Consistently, ~2.5% of the clones recovered from each of the *Arabidopsis* lines and from the maize *wx-m7* allele are +0 excisions.

Differences in the frequency with which we recover small footprints (e.g., +2, +1 or –1) and deletions beyond the target site duplications argue that there may be subtle differences in the host DNA repair machineries of maize and *Arabidopsis* or differences in how that machinery interacts with *Ac* transposase. These differences are probably not due to the deletion in the *AcΔNael* transposon: the sequences deleted are not required for transposition (Coupland *et al.*, 1988) and the deletion does not alter any putative transposase binding sites (Chaterjee and Starlinger, 1995; Kunze and Starlinger, 1989, Wang *et al.*, 1996). It is tempting to speculate that, because *Ac/Ds* elements have been active in the maize genome for thousands of years, maize may have evolved features of DNA repair following *Ac/Ds* excision that other species have not. This adaptation to the presence of *Ac* is also supported by studies showing that *Ac* is generally more sensitive to the negative effects of gene dosage in maize than it is in other species (Dean *et al.*, 1992; Keller *et al.*, 1993; Swinburne *et al.*, 1992).

Little is known about the plant genes involved in DNA repair of *Ac/Ds* excisions; however, the end-joining of *Ac/Ds* footprints contrasts sharply with the gene conversion/gap repair following excision by P elements in *Drosophila* and *Tc1* elements in *Caenorhabditis* (Engels *et al.*, 1990; Gloor *et al.*, 1991; Kaufman and Rio, 1992; Plasterk, 1993). Plants may carry out similar gene conversion repair of some transposon excision sites, but appear to do so selectively. For example, excision by maize *Mutator* elements may be repaired by both an end-joining mechanism

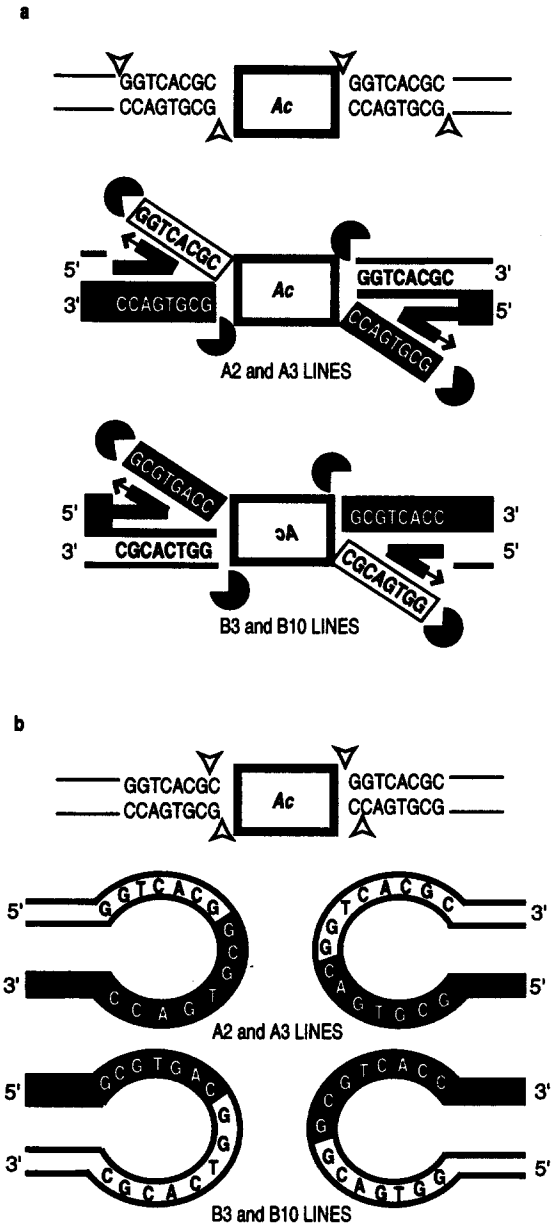


Figure 4. Footprint intermediates for the A2/A3 lines and B3/B10 lines using the two proposed models.

(a) The exonuclease model predicts identical 5′ overhang sequences but on opposite sides and strands when comparing lines A2/A3 and B3/B10. *Ac* must persist at the site during repair to explain the template switching. Repair synthesis and template switching are shown by bent arrows and 5′ to 3′ exonuclease represented by 'Pac-man' symbols (after Saedler and Nevers, 1985)

(b) The endonuclease model, in which the element is removed immediately and DSB ends self-ligate into hairpins. Comparing A2/A3 and B3/B10 lines, the hairpins contain identical sequences 5′ to 3′ but are on opposite sides of the DSB relative to flanking *SPT* sequences (after Coen *et al.*, 1986).

and a gene conversion type mechanism, each used at different points during development (Donlin *et al.*, 1995; Hsia and Schnable, 1996; Lisch *et al.*, 1995). Internally deleted transposons with breakpoints resolved in short regions of homology ('interrupted gap repair') are important evidence for the gene conversion repair mechanism (Hsia and Schnable, 1996); interestingly, such deletions are also common among defective *En/Spm* elements (Gierl, 1996), but not among *Ds* elements (Kunze, 1996).

One explanation for the preferential end-joining of *Ac/Ds* footprints may be that during *Ac/Ds* excision sequentially formed single-strand breaks occur in the host DNA instead of DSBs; thus, DSB repair is never triggered. Alternatively, the gene conversion/gap repair mechanism may require interaction between host products and specific transposon products that *Ac* does not make. Whether the repair mechanism used is determined by the element, the host or both, and what those determinants are remains unclear.

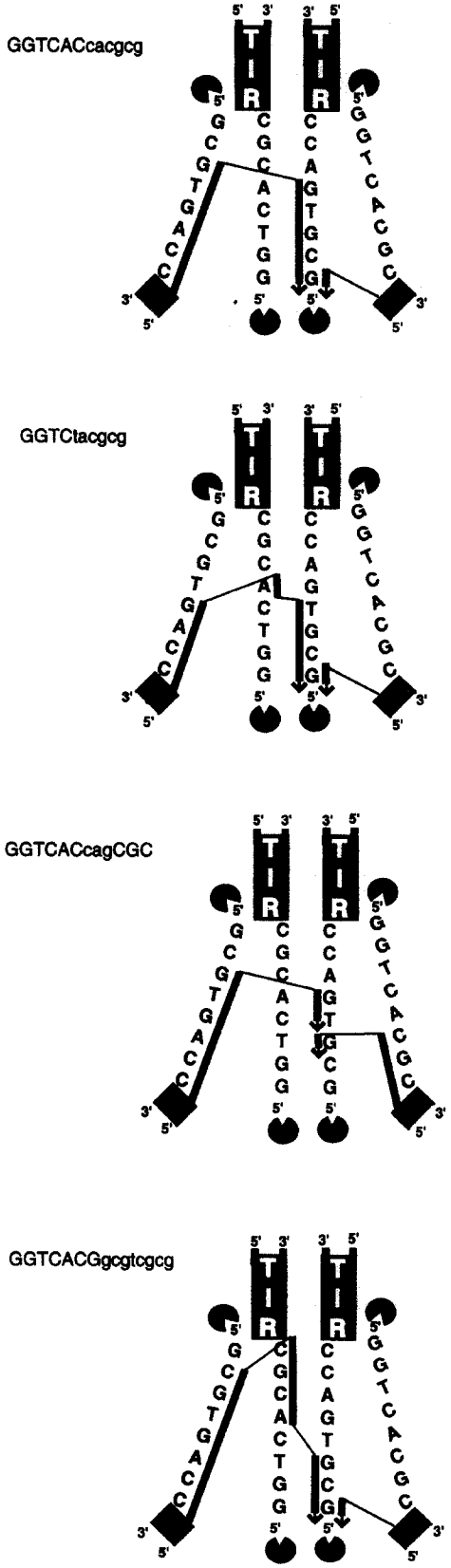
+0 excision

It has been suggested that +0 excisions are formed differently from footprints, and that the two mechanisms may operate in parallel or have common features (Dennis *et al.*, 1986; Scott *et al.*, 1996). The two most likely mechanisms for +0 excision are transposon stimulated recombination between the flanking 8 bp target site duplications (Athma and Peterson, 1991; Lowe *et al.*, 1992; Puchta *et al.*, 1993; Puchta *et al.*, 1996; Tovar and Lichtenstein, 1992; G. Shalev and A. Levy, unpublished) or annealing of complementary host DNA overhangs following transposon removal (Saedler and Nevers, 1985; Schiestl and Petes, 1991; Scott *et al.*, 1996). Both models depend on the presence of direct repeats flanking the transposon; we would predict that +0 excision should not occur from non-identical flanking DNA.

Both maize and *Arabidopsis* yielded comparable, low frequencies of clones with +0 excisions. In maize, +2 and +1 footprints are formed much less frequently than either +3 or +0 products, suggesting +0 excisions do not result from a progressive loss of bases that go from +2 to +1 to +0. *Arabidopsis* also consistently yields ~2% of clones with +0 excisions but, unlike maize, low frequencies of +2, +1 and −1 footprints also occur (Figure 3); thus, the data are inconclusive with regard to the mechanism of +0 excisions.

Implications for models of footprint formation and transposition

Two models for footprint formation by end-joining have been proposed (Coen *et al.*, 1986; Peacock *et al.*, 1984; Saedler and Nevers, 1985), and one, the 'exonuclease model', requires that the *Ac/Ds* element remain at the donor site during the repair process (Figure 4a) (Peacock *et al.*, 1984; Saedler and Nevers, 1985; Scott *et al.*, 1996). Staggered nicks are made by transposase at each end of the target site duplications, similar to those created on transposon insertion; whether nicks are made on both



strands at the same time or sequentially is unknown. Eight bp 5′ overhangs remain attached to the *Ac/Ds* element and repair synthesis is initiated at the free 3′ ends in the host DNA. Exonuclease activity may act at exposed 5′ ends, creating the short deletions present in many footprints. The repair synthesis does not proceed into the transposon (why is unclear) and instead can template switch to the 5′ overhangs (Figure 4a), explaining the inversions and complementary bases found in typical footprints. Once the transposon is removed, the remaining ends are rejoined and any gaps or mismatches repaired.

In the alternative 'endonuclease model', based on studies of the *Tam3* transposon (Coen *et al.*, 1986; Figure 4b), excision is initiated by staggered nicks separated by one bp instead of eight, the transposon is removed immediately and the DSB termini are covalently sealed into hairpin structures. These hairpins are randomly nicked by a single-strand endonuclease, the rearranged termini rejoined and any remaining gaps or mismatches repaired. Exonuclease activity on free 5′ ends during the nicking and religation process may also contribute to rearrangements and deletions found in typical footprints (see Coen *et al.*, 1989).

We propose modification of these models to account for footprint sequences we have recovered from maize and *Arabidopsis*. Eight of the footprint sequences in Figures 2 and 3 (a total of 11 clones from three different *Arabidopsis* lines and from maize) can be formed by the exonuclease model but not by the endonuclease model. Furthermore, there are four footprint sequences that cannot be explained by *either* model as currently proposed (marked by # in Figure 3). While each of these footprints has been recovered only once, the complexity of their sequences and the fact that the only sequence changes found are at the empty donor site suggest they are not PCR errors.

The four indicated footprints can be explained if the exonuclease model is modified to consider all the templates available if element ends are brought together during transposition (Feldmar and Kunze, 1991; Frey *et al.*, 1990) and this structure is considered in three dimensions (Figure 5). In addition, we propose that template switching can occur more than once in the synthesis of any given footprint and appears to move to comparable positions along the eight bp overhang. This modified exonuclease model explains all the *Ac/Ds* footprint data from maize and heterologous hosts. Interestingly, it also explains two unusual footprints left by the *dTph1* element of *Petunia*, which has terminal inverted repeats similar to those of *Ac/*

Figure 5. Modified exonuclease model of footprint formation.
Footprints shown are from Figure 3 and cannot be explained by either the exonuclease or endonuclease models as currently proposed (Coen *et al.*, 1986; Saedler and Nevers, 1985). Each footprint is shown with the proposed events by which it formed. DNA repair synthesis is shown as heavy black arrows.

Ds and which also creates eight bp target site duplications (Renckens *et al.*, 1996).

A modified endonuclease model can account for the 12 footprint sequences marked in Figures 2 and 3 if (i) the one bp staggered nicks that initiate transposition can occur at bases other than those immediately adjacent to the transposon, (ii) hairpins can form, open, reform and reopen prior to ligation and (iii) untemplated nucleotides can be added by a terminal transferase enzyme (as in V(D)J rearrangement; Boubnov *et al.*, 1993). We note, however, that these changes allow, in principal, any footprint to arise from any starting sequence.

Finally, as in maize, all the footprints we recovered from *Arabidopsis* are consistent with deletion or replacement of the base closest to *Ac*, perhaps as an obligate part of the transposition mechanism (Renckens *et al.*, 1996, Scott *et al.*, 1996). For example, the +14 footprint from *Arabidopsis* plant B10-l, 'GGTCACGgcgtgacc -GTCACGC' (Figure 3), clearly shows loss or change of the nucleotide closest to the transposon on each side even with the apparent addition of a complete, inverted, target site duplication in between. This deletion or replacement is unambiguous in most cases and cannot be ruled out in the remainder. Removal of one base in this way might be expected from the endonuclease model, but is an unexpected aspect of the exonuclease model and may have implications for the *Ac/Ds* transposition mechanism.

Experimental procedures

Maize DNA

Maize plants containing the *wx-m7* allele were grown in a greenhouse until four weeks old, severed at the base and immediately frozen in liquid nitrogen for DNA extraction by a CTAB extraction protocol (Doyle and Doyle, 1988).

Arabidopsis *lines*

The A2 and A3 *Arabidopsis* lines were derived by transformation with pCL02213; the B3 and B10 lines were derived by transformation with pCL0383. Both plasmids and all four transformant lines have been described previously (Bhatt *et al.*, 1996; Lawson *et al.*, 1994). Plants were incubated in growth chambers at 22°C and 50–70% humidity under continuous light. Mature plants, approximately 20 cm tall, were harvested for DNA extraction by severing at the base, freezing immediately in liquid nitrogen and storing at –80°C. Genomic DNA extraction was carried out using the same CTAB extraction protocol (Doyle and Doyle, 1988).

Footprint collection

Transposon footprints were amplified from DNA of individual *Arabidopsis* plants using 4 ng of genomic DNA template in 100 μl PCR reactions. The *C15* and *B37* PCR primers (Bancroft *et al.*, 1992) were used to amplify a 540 bp fragment from those template molecules lacking *Ac* (Figure 1). Amplification using the *Ac3'* primer (5'GGAATTCGTTTCCGTCCCGC3') in conjunction with either *B37* or *C15*, depending on the orientation of the *Ac* element, was used to verify that these plants still carried an *Ac* element inserted in the *SPT* marker (Figure 1). In maize, the *Ac3'* primer in conjunction with *131* was used to verify the presence of *Ac* upstream of *wx* in each plant.

PCR amplification was carried out by hot start using Amplitaq Polymerase (Perkin/Elmer, Norwalk, CT). PCR reactions were subjected to 40 cycles of 1 min at 95°C, 1 min at 58°C and 3 min at 72°C, one cycle of 10 min at 72°C and then held at 4°C. Amplified product from three independent PCR reactions per plant was combined, precipitated, digested with *Bam*HI and *Xho*I, gel purified and the 200 bp fragment directionally cloned into pUC119. *E coli.* DH5α cells were transformed and plated on LB plates containing ampicillin, IPTG and X-Gal (Sambrook *et al.*, 1989). Approximately 100 random light blue and white colonies were purified for each plant, plasmid DNA prepared by alkaline lysis minipreps (Sambrook *et al.*, 1989) and restriction mapped.

Sequencing

Double-stranded plasmid DNA was alkaline denatured and sequenced by the dideoxynucleotide method using Sequenase 2.0 (US Biochemicals, Cleveland, OH). 7-deazaguanosine triphosphate was included in the labeling step to eliminate polymerase pauses due to secondary structure. Sequencing reactions were separated on manual 6% acrylamide gels and visualized by autoradiography.

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